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A SYSTEM FOR EXPOSURE OF RATS AND MICE TO AEROSOLS OF PLUTONIUM--ETC(U)

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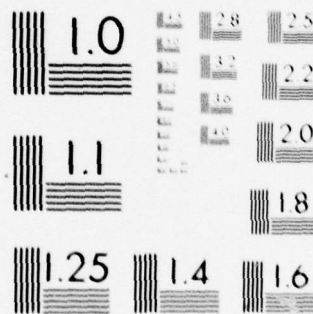
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A SYSTEM FOR
EXPOSURE OF RATS AND MICE TO AEROSOLS
OF PLUTONIUM OXIDE

2. DESIGN AND CONSTRUCTION OF AN
EXPOSURE SUITE FOR MICE

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M. WALSH, A. BLACK, S.R. MOORES AND J. FRITCHARD

ABSTRACT

A system is described for the administration of plutonium oxide aerosols to mice, and for subsequent handling of the exposed animals.

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INTRODUCTION

Studies are being made to investigate the early and late pathological effects of plutonium oxide (PuO_2) deposited in the rodent lung and their dependence on particle size and dose. The work is being carried out in collaboration with the Department of Radiobiology at The Medical College of St. Bartholomew's Hospital, and is partly supported by Euratom.

The primary design consideration was the safety of personnel involved in the experiments and consequently all operations associated with animal exposures are carried out in glove boxes under carefully controlled conditions. This has placed restraints upon the manner in which these operations are performed. Thus, equipment has been designed to operate under these conditions and to minimise the possibility of an accidental exposure to ^{plutonium oxide,} PuO_2 .

GENERAL DESCRIPTION OF THE EXPOSURE SUITE

Ventilation

The ventilation system was designed to ensure that the main experimental area containing the glove boxes is maintained at a lower pressure than its surroundings, to minimise the spread of any possible airborne contamination. Clean air from a plenum (P in Fig.1) in the ceiling of the entrance vestibule flows through the clean and dirty change areas into the experimental area from where it is removed via the fume-cupboard. The wall containing door (S) is largely open-work above door level to allow free circulation of air if this door is closed. If an airborne contamination problem develops, the sliding door (X,X) adjacent to the change barrier may be closed, thus creating a relatively high pressure area on the clean change side.

Adjacent to the fume-cupboard is a hatchway (H) opening into a second experimental area which is also equipped with fume-cupboards for the dissection of exposed animals. Beyond the second area is an animal holding room with its own temperature controlled plenum and extract system capable of maintaining the room at 21°C with about 20 air changes per hour.

Protective clothing

Clean overshoes, contact shoes, laboratory coats and gloves are available at the change barrier. Overshoes and coats are worn at all times in the experimental area. These are returned to the dirty change area after use where provision is made for their disposal if necessary.

Four air-line hoods (AH) and two self-powered respirators are stored in the change area for use during any operation which could result in a release of contamination (e.g. glove changing).

Monitoring facilities

A continuous plutonium-in-air monitor (Eberline Mod. α -3) is located near the fume-cupboard where its readout is visible to personnel working in the area. A remote alarm (RA) is located over the main door (D) to alert staff to possible airborne contamination problems before they enter the suite. Back-up estimates of airborne contamination are provided by a continuous air sampler (L.30 Anglepoise). Spot samples are taken during animal exposures and weekly samples at other times. Portable alpha monitors (Type 0339-AP2) are available within the experimental area and an alpha contamination monitor (Type 1828-AP2) at the change barrier for use by staff leaving the suite.

Decontamination facilities

Provision for washing has been made in the clean change area. In the event of excessive personal contamination, a shower is also available, with access from the dirty change area, and an exit into the clean change area.

MAIN FEATURES OF THE EXPOSURE SYSTEM

A PuO_2 aerosol of predetermined particle size and concentration is produced in the aerosol generation box, and is administered to the animals in the exposure box. After exposure, the animals are transferred through the unloading box into clean cages which are housed in the holding box. Here they remain until the external PuO_2 contamination is reduced to a level which permits them to be handled without significant exposure to personnel.

As that part of the suite designed for the exposure of rats is not yet complete, the subsequent description applies specifically to the mouse exposure line; many facilities will be common to both systems however.

Operation of glove boxes

All the glove boxes (Marine and Industrial Plastics Ltd., Fareham, Hants) are maintained by individual compressed air powered ejectors at between 1.5 and 2 in w.g. below atmospheric pressure. Laboratory air is drawn into the boxes through another absolute filter into a common manifold. The extracted air passes through further absolute filters in a plant room before being exhausted to atmosphere.

Air supplied to the exposure and holding boxes is conditioned in the following manner. Fan assisted heat exchangers, mounted above the boxes, supply air at about 15°C (59°F). These are supplied with cooling water at about 9°C (48°F) which is automatically shut off if the system develops a leak. The air passes through a regulating valve and absolute filter to distribution pipes within the boxes.

In the exposure box the air serves to cool the mice during exposure, its temperature being monitored at the inlet filter (Telemax TC1-II) and above the restraining tubes (Digitron Mod 1751 and TC7 thermocouples). However in the holding box, the air is required to maintain the box temperature at 21°C (70°F). Thus, before entering the box, the air passes through a heater unit controlled by a temperature sensor inside the box (Telemax TC1-II).

The arrangement of the suite services is shown in Fig.2 and a general view of the mouse exposure line in Fig.3.

All glove box posting operations are carried out in the usual manner using standard PVC posting bags which are sealed with a portable R.F. tong welder (Intertherm Type J.P.9).

Aerosol generation box

The system for producing aerosols of PuO_2 has been thoroughly investigated using ThO_2 (Black et al. 1978). Aerosols are generated from aqueous suspensions of sized PuO_2 particles with a Retec nebuliser (Cavitron Corporation, Van Nuys, California). The aerosol passes to the exposure chamber situated in the adjacent glove box through a sealed pipe.

As the aerosol generation box contains pressurised gas lines, provision has been made to shut off supplies automatically if the box pressure rises to 2 in w.g. above atmospheric.

The exposure box

This contains the mouse exposure chamber (see Fig.4) which was designed to accommodate up to 60 mice in plastic restraining tubes so that only the animal's noses are exposed to the aerosol. The chamber consists of a rectangular Perspex box (55 x 20 x 32 cm) containing a second open ended box with its open ends sealed against the end walls of the first box. In this way a 10 l reservoir is formed at the top of the chamber and a 2 l one at the bottom. Blocks are sandwiched between the inner and outer box walls to form ten vertical rectangular channels on each side of the chamber connecting the upper and lower reservoirs. Each channel contains three exposure ports giving a total of sixty in all.

The mouse restraining tubes are sealed in the ports by means of a Chloroprene annulus with an internal diameter of 2 cm. Each annulus is sealed against the face of the chamber by a rectangular section rubber gasket which is compressed by a light alloy ring screwed to the chamber wall. A perforated Perspex plate is located at a distance of 5 cm from the face of the chamber providing support for the restraining tubes. This system gives an airtight seal and, at the same time, permits tubes to be inserted in, and withdrawn from the chamber quite rapidly. The seals can readily be replaced in case of damage.

Aerosol is introduced through the centre of the top of the chamber directly above a horizontally mounted low speed impellor driven by an induction motor.

This disperses the aerosol evenly within the reservoir. The base chamber is connected via an absolute filter to a suction pump. In order to ensure that the aerosol is distributed uniformly between the twenty channels, each channel is sealed across the bottom with Perspex containing a 1.5 mm orifice. The pressure drop across this orifice, being much greater than variations in pressure between the various channels and the exit port, tends to even out variations in flow between channels. A cleanable wire mesh filter is located above each orifice to prevent its occlusion by foreign matter.

The total flow through the chamber is adjusted to give a flow of about 0.5 l min^{-1} in each channel. At this rate it was considered that depletion of aerosol concentration in the lower ports, due to deposition of inhaled particles in animals in the upper ports, would be minimised. In use, the exposure chamber is maintained at approximately 1 mm w.g. below the glove box pressure. This ensures that any small leaks in the system are inwards, thus reducing glove box contamination.

After consideration of the problems of restraining mice during nose-only exposures, the final design of tube adopted is shown in Fig.5. These tubes are injection moulded in clear polycarbonate plastic (Pinza Plastics Ltd, High Wycombe). They have the advantage of being transparent so that it is possible to observe mice during exposure and are disposable avoiding the need for decontamination. They are wide enough to accommodate animals of up to 30 g body weight and sufficiently long to ensure that the operator's gloved hands can obtain a secure grip during loading and unloading. The space behind the mouse is packed with cotton wool and the tube is closed by a rubber bung. After several trials a head cone of 55° was adopted. Too blunt a cone did not locate the animal's head securely enough to keep its nose outside the tube whereas too sharp a cone would be uncomfortable for the mice as it allowed their eyes to come into contact with the inner surface. The diameter of the nozzle was sufficient to allow the mouse's muzzle to protrude into the centre of the aerosol stream.

During experiments, a maximum of six ports is available for air sampling. Four of these are normally occupied by in-line filter holders (Gelman 25 mm diameter) fastened to the open ends of mouse restraint tubes and connected to the conical ends by 6 mm i.d. PVC tubing. Each holder contains a filter paper (Millipore VC 0.1 μ m pore size) and is connected to a common vacuum manifold. Individual filter units are precalibrated on a test rig which relates the pressure drop across the paper to the true flow-rate, at a fixed depression. In use, the depression is monitored on a manometer inside the glove box. Data from these samplers is used to calculate the mean aerosol concentration during exposure.

The remaining two samplers are 7-stage cascade impactors (Mercer et al. 1970) which are attached to Perspex holders and can be inserted directly into the standard mouse positions. These samplers are also calibrated for flow at a particular depression which is monitored during exposures. In order to avoid self-absorption problems when α -counting the impactor stages, samples are only taken for a short period during the exposure. From these counts the activity median aerodynamic diameter (AMAD) of the aerosol may be calculated.

Manometers measure the pressure drop across precalibrated filters to monitor the aerosol diluting and total chamber air flows. The latter filter is situated downstream of the exposure chamber between two absolute filters which serve to remove the remaining airborne particles. The air is then exhausted by a rotary vane pump.

To reduce the number of pipes penetrating the box walls, the manometers, control valves, flow meters and the sampling pump are fitted inside the exposure box. All gas lines which do pass through the enclosure are protected by absolute filters.

Attached to the exposure chamber are two perforated copper tubes directing the cooling air between the front face of the chamber and the tube support plate, and thus over the surfaces of the mouse restraining tubes. Situated above each

bank of tubes is a miniature thermocouple (Labfacility TC7) measuring the temperature of the outflowing cooling air and these, together with eight further thermocouple sockets are connected to a selector switch and the digital thermometer (Digitron 1751). These sockets are to accommodate the thermocouples fixed inside selected restraining tubes to monitor the temperature of mice during exposure.

Before each exposure, animals are loaded into tubes in the animal room, passed through the hatchway and transferred into the exposure box through a specially designed 'sphincter valve'. This valve consists of a series of concentric annular rubber flaps which permit the transfer of one tube after another through the box wall and at the same time prevent any leakage from the box. The opening is protected when not in use by pivotted sealing flaps on both sides. This procedure has proved to be quick and efficient in its use, taking less than 15 min to load and insert a full complement of animals.

On occasions, it is necessary to kill some animals immediately post-exposure. Initially sodium pentobarbital injection was used but this technique was abandoned because of the dangers involved in using a hypodermic syringe in a glove box. Consequently, a simplified version of the exposure chamber containing 12 ports was constructed and fitted into the exposure box. The mice are killed whilst inside the restraining tubes using nitrogen gas. With a high flow-rate of this gas through the chamber, animals succumb quickly and without undue distress.

Extra glove positions have been fitted to permit installation and maintenance work on the aerosol and cooling air pipework and thermocouple wiring, all of which are at roof level in this glove box. The fully equipped box is shown in Fig.6.

The unloading box

After exposures animals are transferred in their restraining tubes to the unloading box where the bungs, packing materials and tubes are discarded, and

the animals placed in transfer cages. When full, each transfer cage is passed through a large diameter tunnel into the holding box. Trash can be removed from the unloading box through a posting port in the rear wall.

The holding box

In this box animals are transferred into cages (North Kent Plastics, Dartford) fitted in storage racks capable of holding 120 animals. Water bottles are filled from a syphon in the roof of the box with a reservoir and valve outside, and may be emptied using a similar system in the floor of the box, which discharges directly into a liquid waste carboy. Bedding changes and servicing of food and water supplies may be performed as required, and a small vacuum cleaner is provided so that a suitable standard of cleanliness can be maintained.

This glove box has been fitted with extra glove sets at high level to facilitate handling cages at the top of the storage rack. Also incorporated are extra posting ports and a special large diameter port to permit the passage of cages.

During the time the animals are in this box (7-10 days) they will clean much of the surface contamination off their pelts by preening, the ingested material being removed in faeces. Thus, when they are removed from the box and transferred to the animal room, there is much less chance of particulate material being resuspended from their pelts into the room atmosphere. Initial operations in the animal room are nevertheless performed whilst wearing airhoods, and appropriate air samples are taken.

The sampler service box

This glove box is mounted on the rear face of the aerosol generation box and is reserved for the loading and unloading of the Mercer impactors. Lightweight gauntlets, overhead illumination and a matt black floor have been provided to facilitate handling the small component parts of the impactors.

The sample counting box

After unloading, the filter papers and Mercer impactor slides may be posted

into this glove box and counted in an alpha-counting head (Type 1588A). The associated electronic rack is mounted underneath the glove box.

COMMISSIONING TRIALS

Cooling system

It is necessary to avoid exposing the mice to stress induced by a rise in body temperature while in the restraining tubes. The forced draught cooling system on either side of the exposure chamber was designed to maintain satisfactory conditions in this respect. During experiments the temperatures of up to 8 mice can be monitored using the thermocouple system described earlier.

The temperatures of mice in cooled and uncooled tubes in the bottom port positions are shown in Fig.7. It can be seen that for uncooled tubes (cooling air switched off) the temperatures produced were relatively high. The cooling air produced a temperature reduction of about 6°C within the restraining tubes. In general, tube temperatures declined during the 30 min exposure.

Temperature readings were taken in two sets of tubes, one mounted nearest the cooling air outlets (bottom ports), the other set furthest away (top). After a time, the tube positions were reversed and more readings taken. Some typical results are shown in Fig.8. These indicate that the cooling effect is most pronounced for the bottom ports, but even at the furthest position from the cooling air outlets, tube temperatures were below 30°C . The reduced cooling effect at the top positions was small in comparison to the variations found between animals. Furthermore, with exposures of 30 min no animals have exhibited symptoms of distress.

Exposure chamber distribution trials

A series of experiments have been performed using ThO_2 aerosols to establish if the distribution of aerosol was uniform between the various ports. Samples were taken at several ports in each face of the chamber, and the mean concentration of each face compared, thus giving a "front"/"back" ratio. This varied from 0.91 to 1.13 with a mean ratio for several runs of 0.99 ± 0.07 .

This indicates that there was no bias from one side of the chamber to the other. The aerosol concentration at various positions in the chamber ranged from $\pm 3-5\%$ of the mean value, with an average spread of 4%.

During routine exposures, the aerosol was sampled at four positions in the centre row of ports. The aerosol concentrations derived from these were within $\pm 8\%$ of the mean value, and the range over several runs was 3-11%.

Exposure chamber aerosol depletion tests

To investigate the effect of mice in the upper ports exhaling depleted aerosol into the channels thus reducing the concentration at the lower ports, the lung burdens of 170 mice exposed to PuO_2 have been examined. Mice were classified as being exposed in the bottom ports, only if the two higher ports were occupied by other mice. If one of the upper ports was occupied by a sampler, the mouse at the lowest port was classified as belonging to the middle group. In order to compare groups with different lung burdens, the individual results were normalised by calculating the percentage spread about the mean lung burden of each exposure group. The results are summarised in Table 1.

The animals exposed in the bottom ports exhibited lung burdens significantly lower than those in the other two positions ($p < 0.05$). This effect is probably due to the depletion of the aerosol by the other animals. This difference, however, was not felt to be important when the range of lung burdens in any one row is compared to any other (Black et al., in press).

Aerosol resuspension in exposure system

Tests have been carried out to determine whether any resuspension of particles previously deposited in the exposure system occurs. The experimental evidence to date suggests that no such effect exists, but the pipe connecting the aerosol generation system to the exposure chamber has been made readily detachable, and may be decontaminated along with the exposure chamber, in the exposure box should this prove necessary.

Aerosol resuspension in holding box

Measurements have been made after runs to determine if any PuO_2 particles are resuspended into the holding box air from contaminated bedding, pelts or faecal matter to be possibly reinhaled by the animals. Air samples were taken using a personal air sampler mounted half-way up the cage rack directly over the cages situated at the lower levels. The samples ran for 6-8 hours at a sampling rate of 2 l min^{-1} . The results suggest that resuspension of PuO_2 particles will account for less than 0.1% of the dose intentionally achieved during exposure.

SUMMARY

A system has been described for the administration of plutonium oxide aerosols to mice in order to study the pathological effects in the lung. Although the part of the system for exposing rats has not yet been completed, the mouse exposure line has been successfully used for some time, and no major problems are anticipated in completing the suite. A largely empirical account of the development and construction of the suite has been given. The specific details of the preparation of PuO_2 , proving the sampling systems and the experimental results have been, or will be described elsewhere.

ACKNOWLEDGEMENTS

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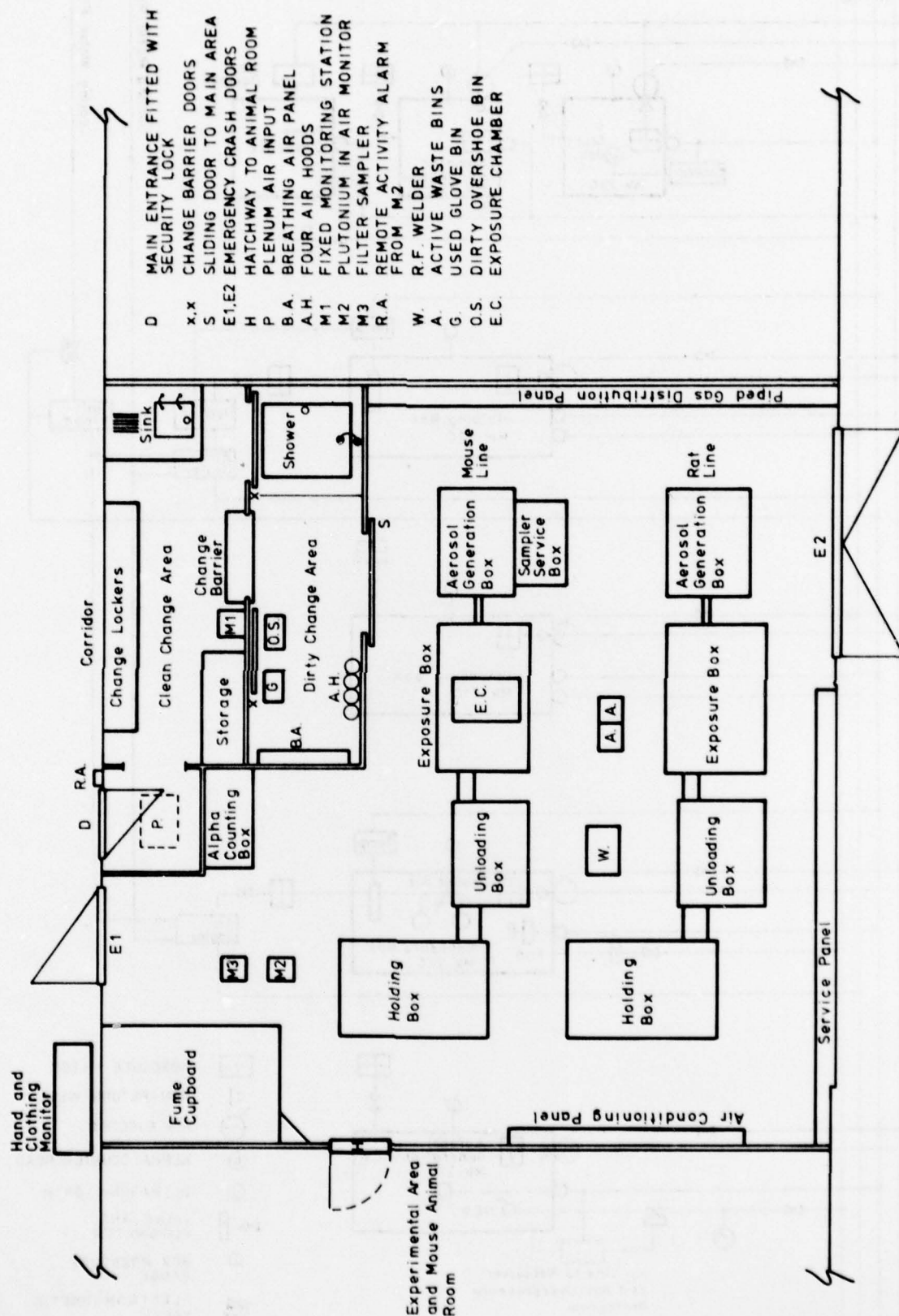
MERCER, T.T., TILLERY, M.I. and NEWTON, G.J. (1970)

A multistage low flowrate cascade impactor.

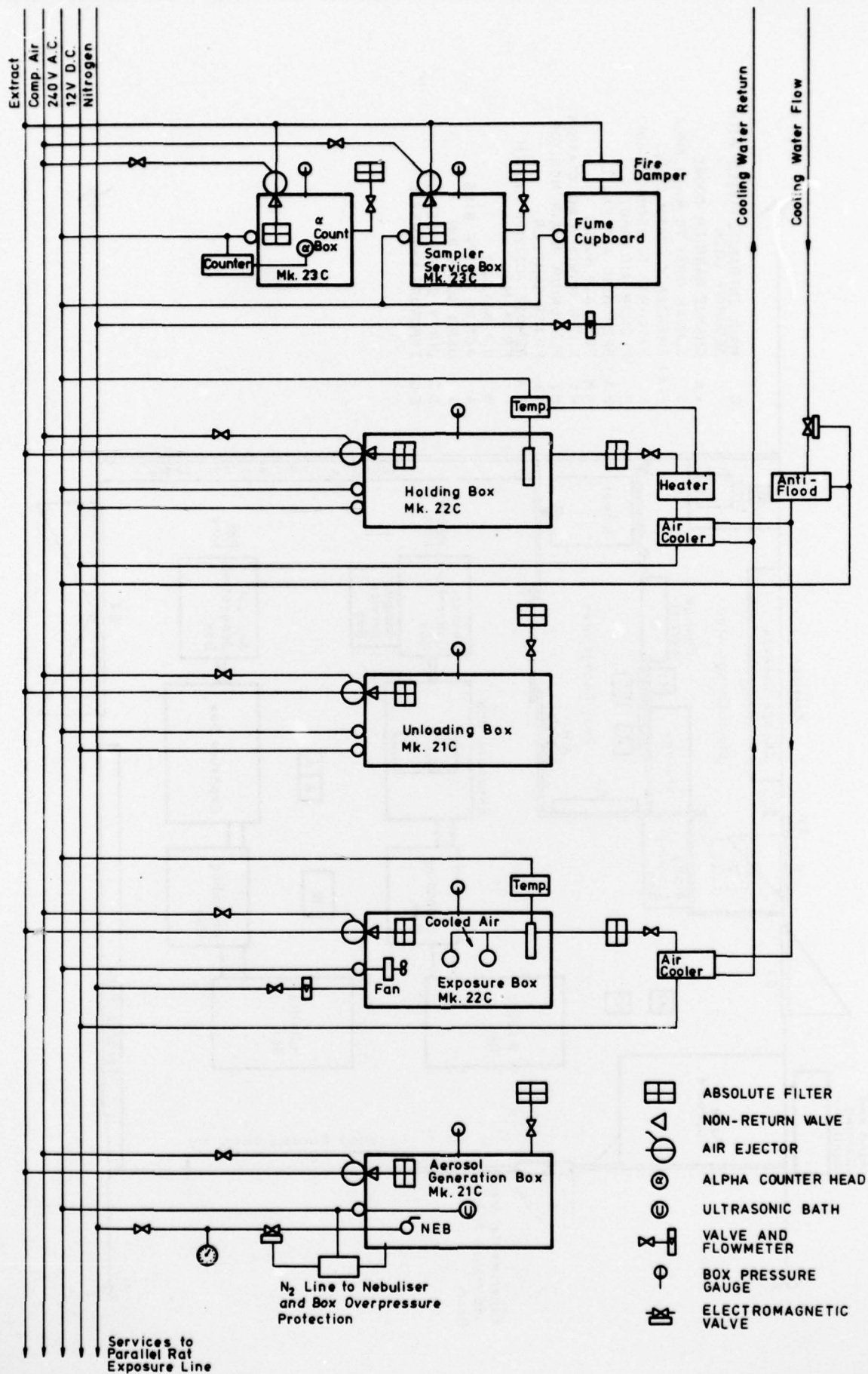
J. Aerosol Sci., vol.1, p.9.

Position in Chamber	Lung Burden Normalised %	Standard Deviation	Number of Observations	Standard Error
Top	104.5	23.4	71	2.79
Middle	103.1	26.2	62	3.37
Bottom	85.3	18.9	37	3.15

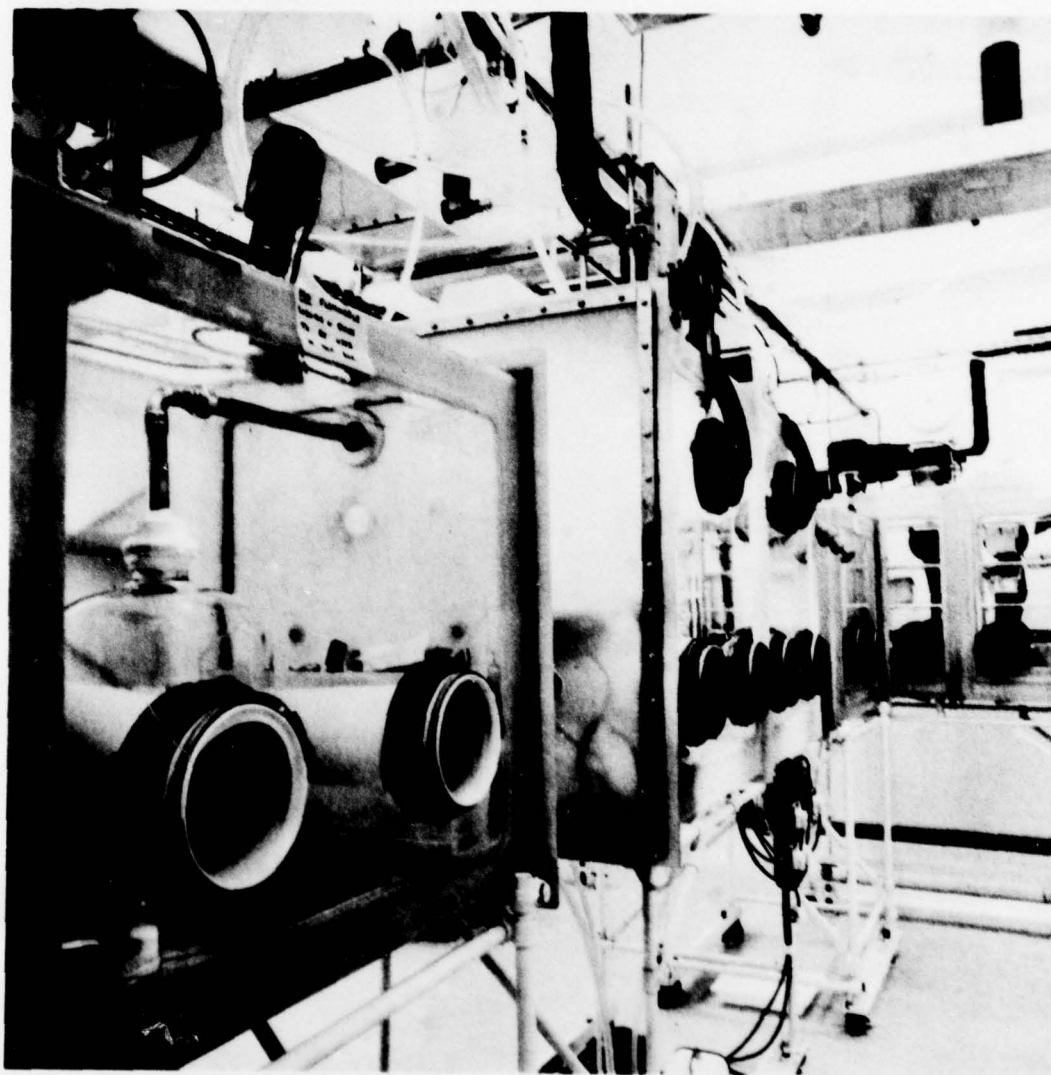
A.E.R.E. R.9251. TABLE 1. EFFECT OF AEROSOL DEPLETION IN
PuO₂ EXPOSED ANIMALS



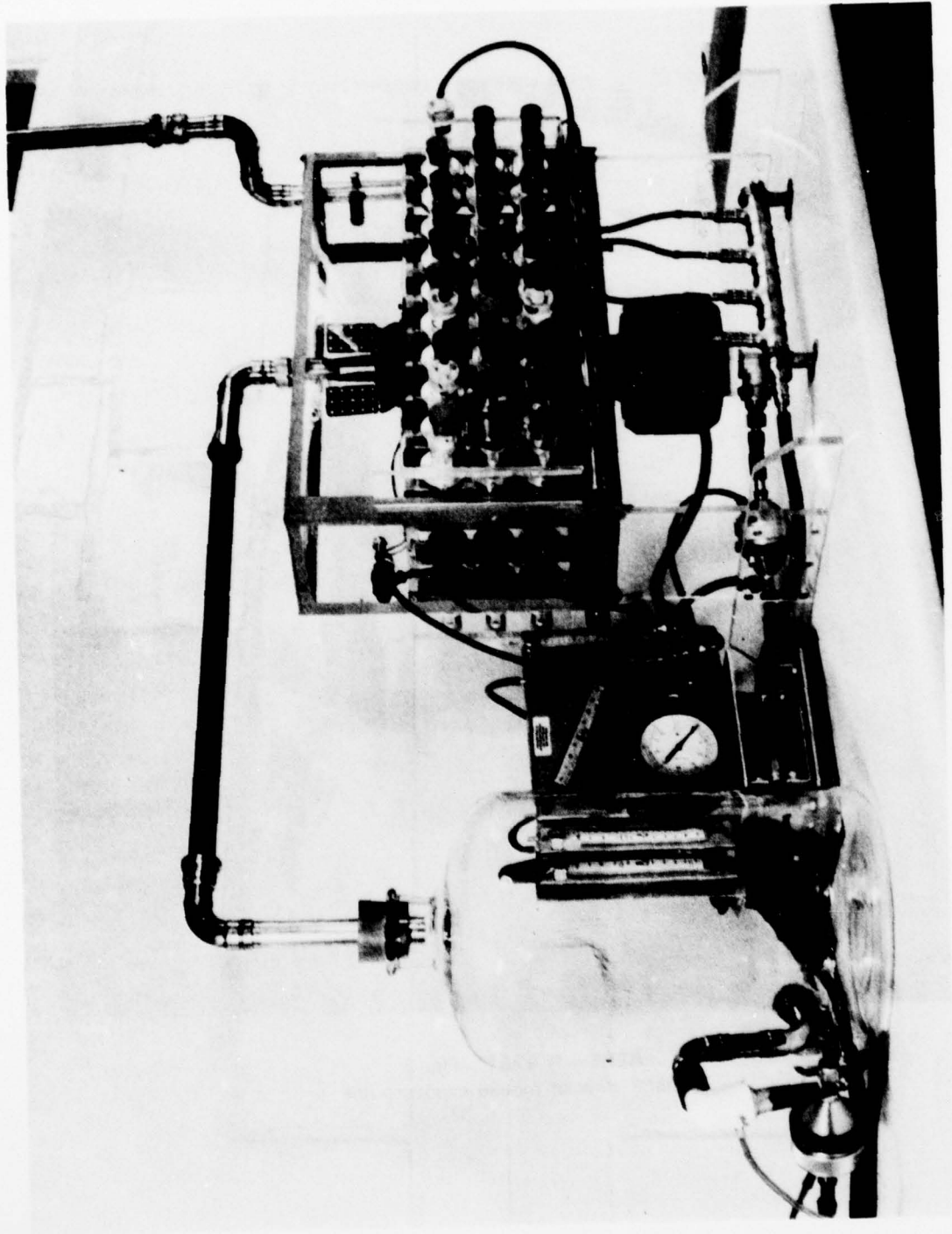
A.E.R.E. R.9251.FIG.1. OVERALL LAYOUT OF SUITE



A.E.R.E. R.9251.FIG.2.ARRANGEMENT OF SERVICES

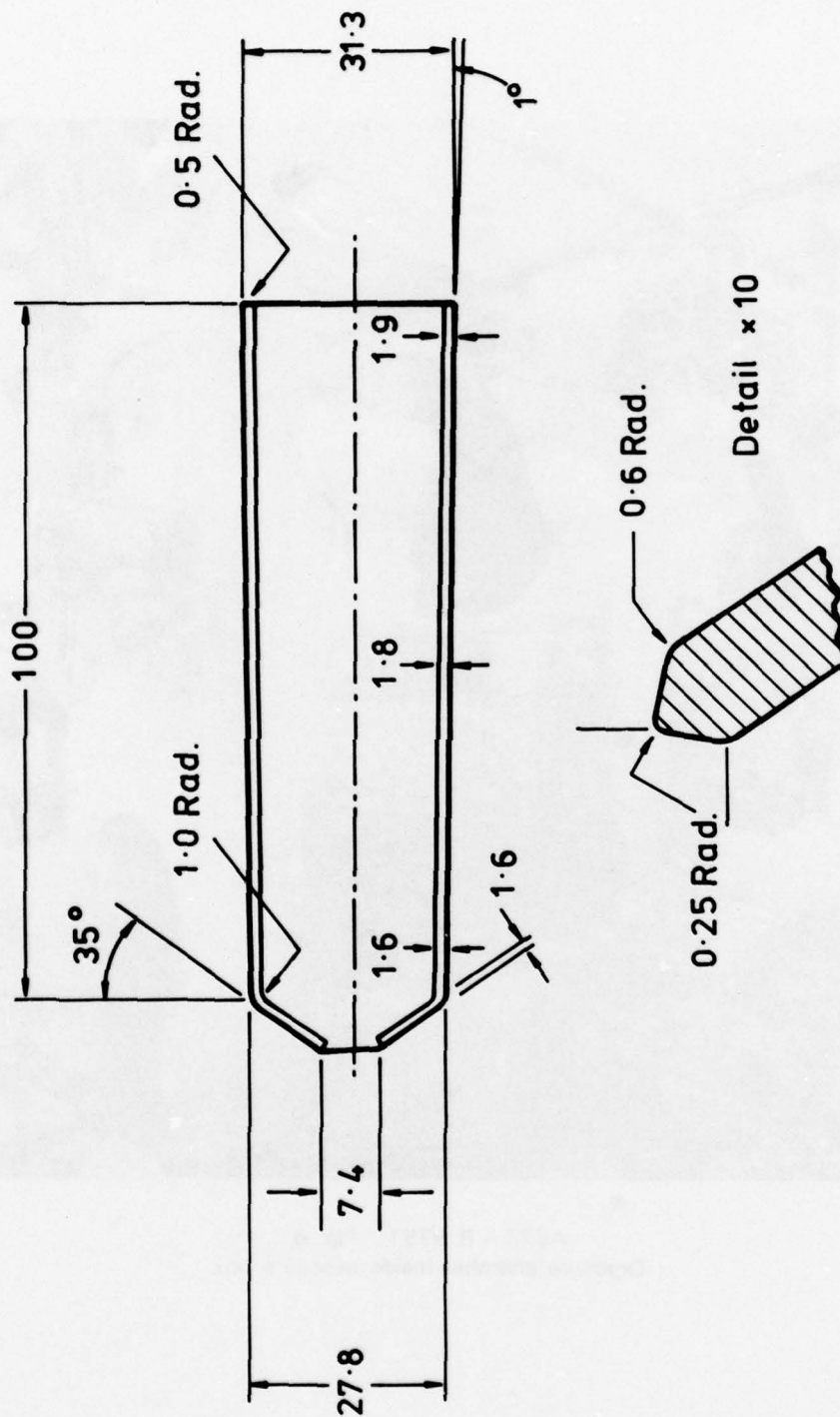


AERE - R 9251 Fig. 3
General view of mouse exposure line

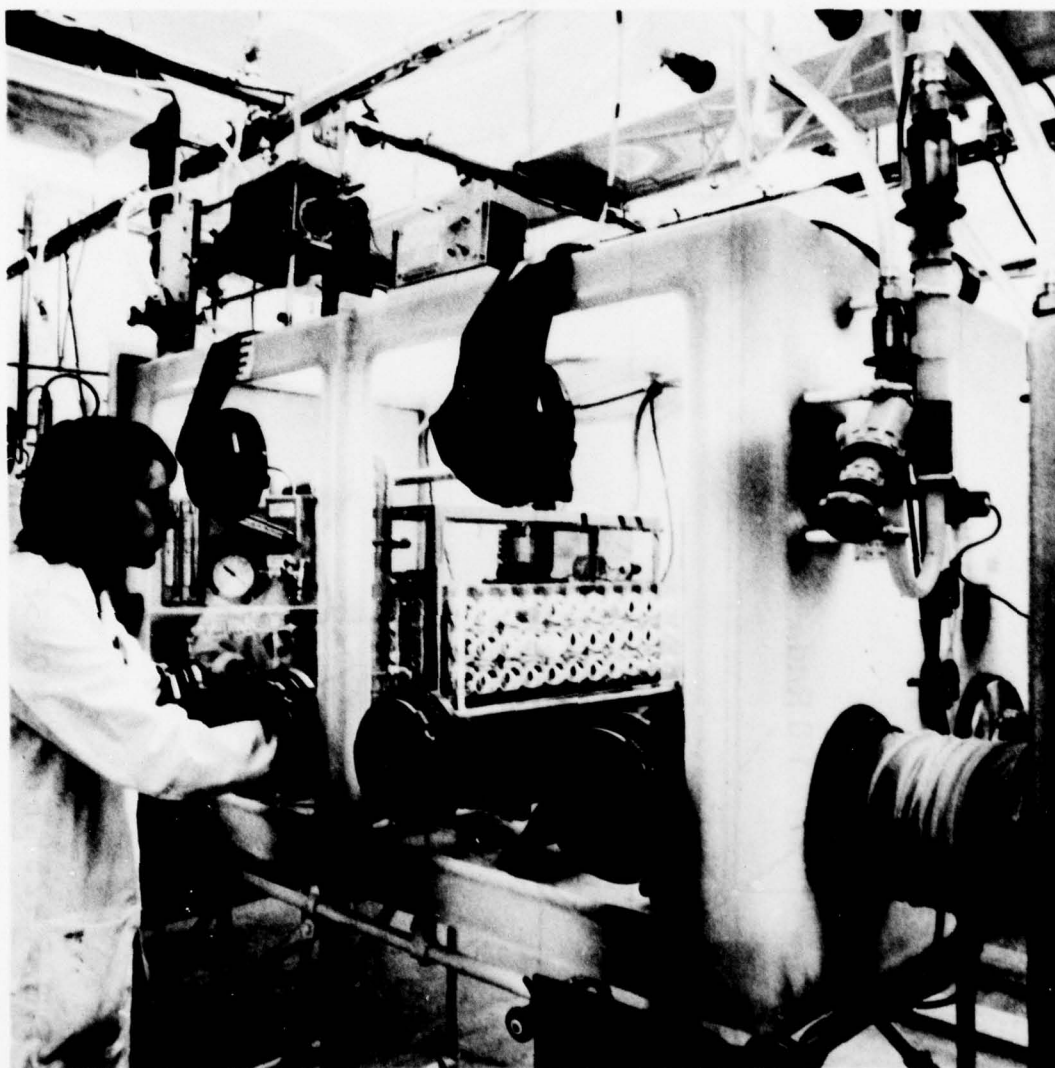


AERE - R 9251 Fig. 4
Exposure chamber and aerosol generation system

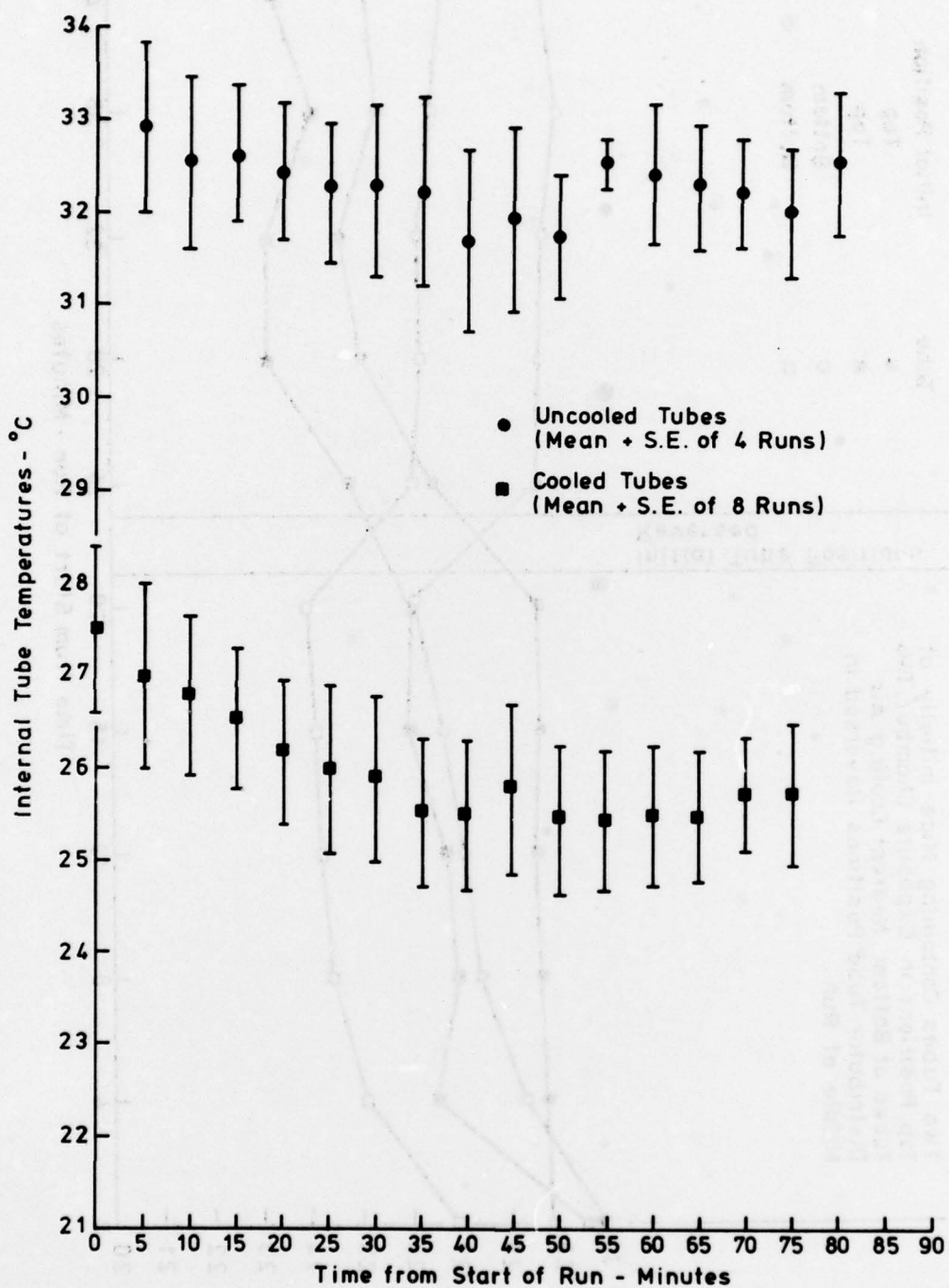
Dimensions in mm.
Material - Clear Polycarbonate



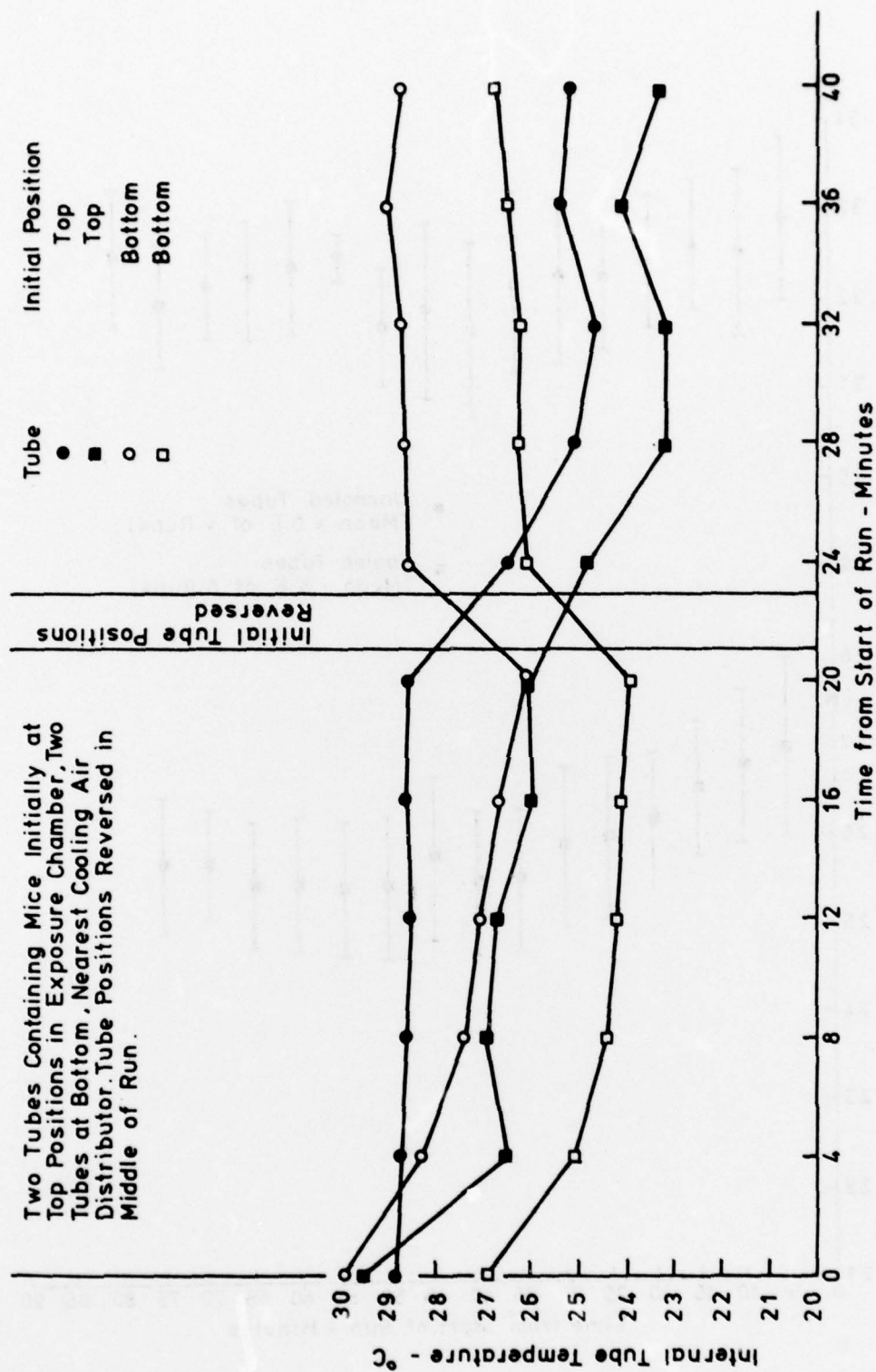
A.E.R.E. R.9251. FIG.5. MOUSE RESTRAINING TUBE.



AERE - R 9251 Fig. 6
Exposure chamber inside exposure box



A.E.R.E. R.9251.FIG.7.EFFECT OF COOLING AIR ON TUBE TEMPERATURES



A.E.R.E. R.9251.FIG. 8.VARIATION IN COOLING EFFICIENCY WITH POSITION IN EXPOSURE CHAMBER